Development and histological characteristics of neurosecretory cells in the cerebral ganglia of *Achatina fulica* (Bowdich)

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Study of postembryonic development of neurosecretory cells in the cerebral ganglia of *Achatina fulica* showed that they first appear in two-month-old snails. The cells are located at the periphery of the procerebrum of cerebral ganglion. The number and size of the neurosecretory cells increase with increasing age of snails, reaching a maximum in eight-month-old snails and thereafter remaining constant in nine- to twelve-month-old snails.

Study of the ultrastructure of the cells showed that they each contain a round shaped nucleus with patches of heterochromatin and a conspicuous single large vacuole in the cytoplasm. In addition, electron-dense elementary granules with a mean diameter of 1600 A° are found to be associated with an extensive Golgi complex and rough endoplasmic reticulum.

Key words: cerebral ganglia, *Achatina*, neurosecretory cells

Introduction

The occurrence of neurosecretory cells in pulmonate snails was first reported by Gabe (1954). Various types of neurosecretory cells can be distinguished on the basis of stainability of neurosecretory cells to different histochemical methods, such as chrome-hematoxylin phloxine (Gomori, 1941) and paraldehyde-fuchsin (Gomori, 1950). The presence of neurosecretory cells in various ganglia of pulmonate snails had been extensively studied. In the basommatophoran, *Lymnaea stagnalis* (L.), the neurosecretory cells are found in the cerebral, pleural, parietal and visceral ganglia (Boer, 1965; Boer and Joosse, 1975). In other basommatophorans, *Australorbis glabratus* (Say) and *Helisoma tenue* (Philippi), neurosecretory cells are present in the cerebral, parietal and visceral ganglia (Lever *et al.*, 1965; Simpson *et al.*, 1966). In stylommatophoran snails, the neurosecretory cells were reported to be present in the cerebral, buccal, parietal and pleural ganglia as well as in the optic tentacles of *Arion ater* (L.) (Smith, 1967), in the buccal ganglia of *Succinea putris* (L.) (Cook, 1966), in the cerebral ganglia (Kai-Kai & Kerkut, 1979) and optic tentacles of *Helix aspersa* (Müller) (Lane, 1964), and in the optic tentacles of *Archachatina marginata* (Swainson) (Nisbet, 1965), *Arion subfucus* (Draparnaud) and *Limax maximus* (L.) (Pelluet & Lane, 1961) and *Milax* spp. (Draparnaud) (Pelluet, 1964). However, there is no report on the presence of neurosecretory cells in the pedal ganglia of pulmonate snails.

The ultrastructure of neurosecretory cells in the cerebral ganglia was studied in *L. stagnalis* (Boer *et al.*, 1968). There are numerous organelles distributed in the cell cytoplasm *i.e.*, mitochondria, rough endoplasmic reticulum, free ribosomes, polysomes, Golgi apparatus, multivesicular bodies and microtubules. In addition, there are electron-dense elementary granules with a mean diameter of 2000 Aº in the cytoplasm.

Most of the knowledge on molluscan neurosecretory cells has been obtained from studies of basommatophoran snails and some stylommatophoran snails (Boer & Joosse, 1975). However, there is virtually no data on the neurosecretory cells of a stylommatophoran, *Achatina fulica* (Bowdich). Hence, the aim of the present study is to report on the development and histological characteristics of neurosecretory cells in the cerebral ganglia of *A. fulica*.

Materials and methods

The process of breeding culture and environmental conditions, the culturing of parent and young snails and the measurement of growth were carried out by the methods of Upatham *et al.* (1988).

Mature *A. fulica* with a shell length of 7 cm (5–7 months of age) were used for morphological study of the neurosecretory cells. The postembryonic development of the neurosecretory cells in the cerebral ganglia was studied in 10 snails out of snails at monthly age of 0–12. In addition, two-week-old snails were examined.

All snails were anesthesized with 1% nembutal for 1 hour, then their shells were broken, cerebral ganglia dissected out and fixed immediately in Bouin's fluid for 12 hours. The specimens were then dehydrated through a graded series of ethanol, infiltrated with dioxane, and embedded in paraffin. Sections were cut on a rotary microtome at 6 μm thickness, stained with chrome-hematoxylin phloxine (Gomori, 1941) and paraldehyde-fuchsin (Gomori, 1950) and examined in an Olympus Vanox light microscope. In addition, the size and number of neurosecretory cells in the cerebral ganglia were measured and counted.

Cerebral ganglia from mature snails were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4 at 4ºC for 2 hours, washed three times in the same buffer and postfixed in 1% OsO_4 in 0.1 M sodium cacodylate buffer, pH 7.4 for 2 hours. The specimens were washed three times with cold distilled water, stained *en bloc* with 1% uranyl acetate in 0.1 M sodium acetate buffer pH 5.1 at room temperature for 1/2 hour, dehydrated through a graded series of ethanol, and embedded in Araldite 502 epoxy resin. Sections were cut with glass knives on a Sorvall MT 2 ultramicrotome, stained with uranyl acetate and lead citrate, and viewed with a Hitachi H–300 TEM, operating at 75 KV.

Results

Neurosecretory cells in the cerebral ganglia which stain positive with chrome-hematoxylin phloxine and paraldehyde-fuchsin occur in the procerebrum of the cerebral ganglia in snails of various ages. These cells stain blue-black with chrome-hematoxylin phloxine and deep purple with paraldehyde-fuchsin. There are no neurosecretory cells in the cerebral ganglia of newly hatched and one-month-old snails (Fig. 1A). Neurosecretory cells with a mean diameter of 17.1 μm (Fig. 3A) first appear in the
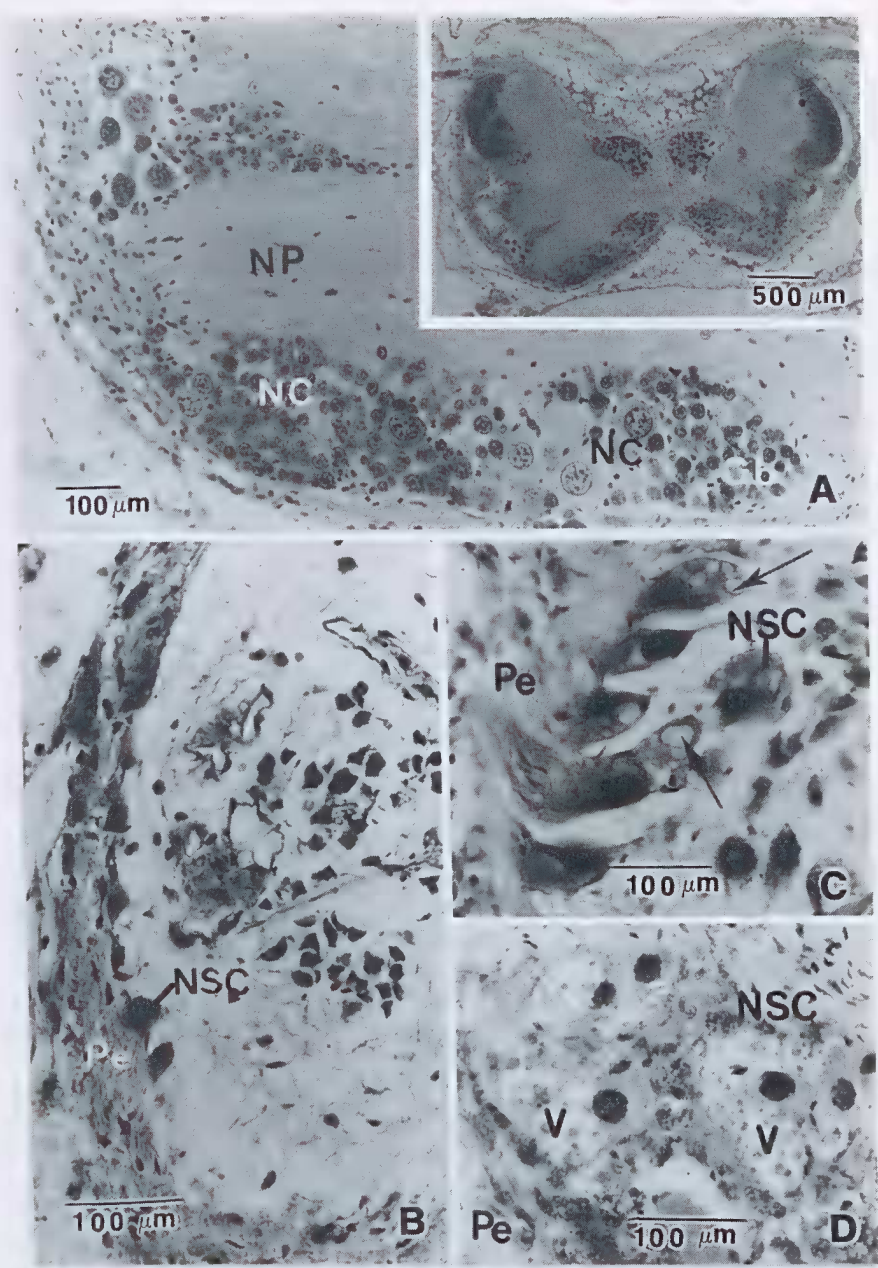
Figure 1. Photomicrographs of longitudinal sections through cerebral ganglia stained with chrome-hematoxylin phloxine. A. High magnification of cerebral ganglion in newly hatched snail showing groups of nerve cells (NC), globuli cells (Gb) and neuropil (NP) of procerebrum. Inset shows overall view of cerebral ganglia in newly hatched snail. B. Cerebral ganglion of two-month-old snail showing neurosecretory cells (NSC) at perineurium (Pe). C. Large neurosecretory cells (NSC) with numerous small vacuoles (arrows) lying against perineurium (Pe) in five-month-old snail. D. Neurosecretory cells (NSC) in nine-month-old snail which contain a conspicuous single large vacuole (V). Pe = perineurium.
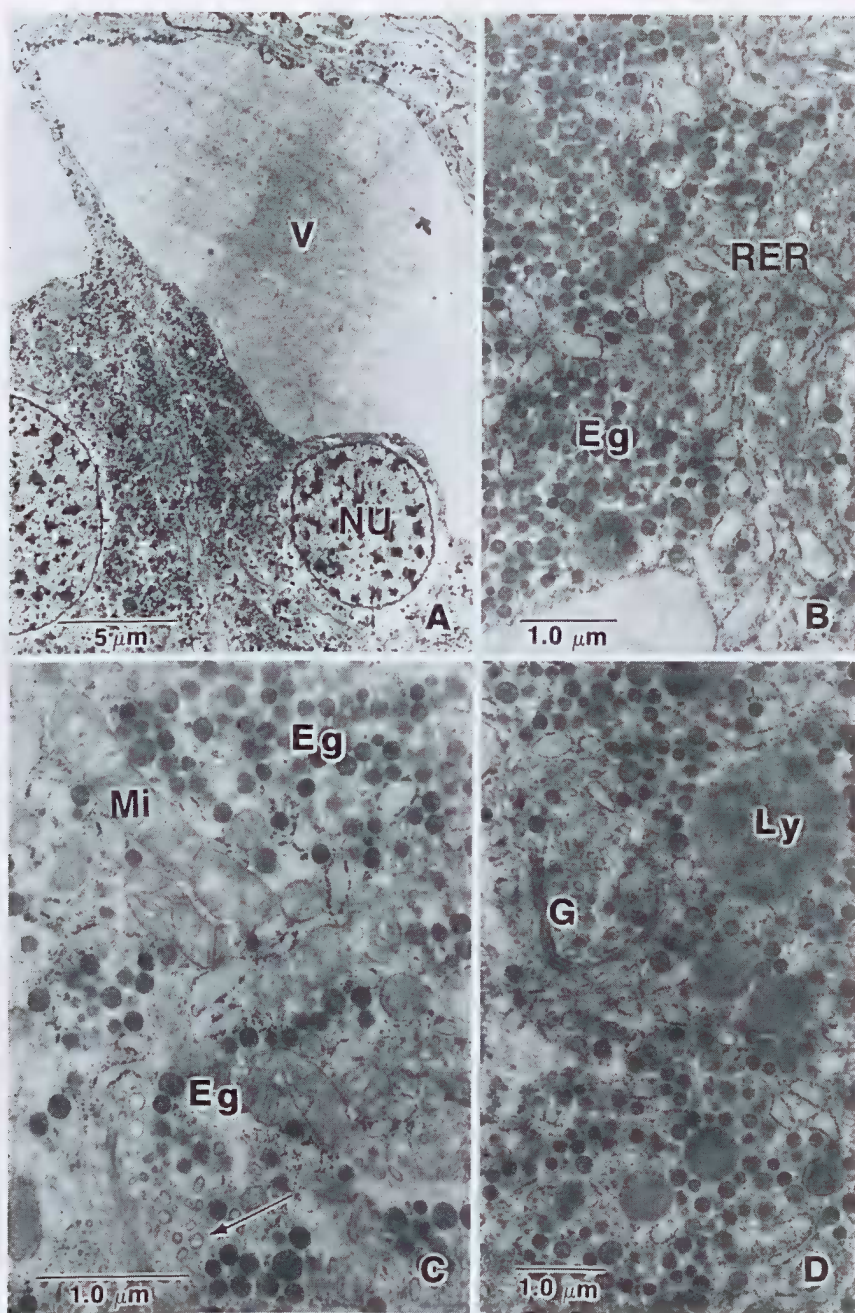
Figure 2.   Electron micrographs of neurosecretory cells in cerebral ganglia A. At low magnification, showing neurosecretory cell with heterochromatic nucleus (NU) and single large vacuole (V). B. Cytoplasm of neurosecretory cell, showing very extensive rough endoplasmic reticulum (RER) and numerous electron-dense elementary granules (Eg). C. Cytoplasm of neuroscretory cell, showing numerous mitochondria (Mi). Electron-dense elementary granules (Eg) are associated with microtubules (arrows). D. Showing many groups of Golgi body (G) and lysosomes (Ly) in cytoplasm.

cerebral ganglia of two-month-old snails. There are nine cells (Fig. 3B) which are located in a single layer at the periphery of the procerebrum (Fig. 1B).

In three-month-old snails, the mean diameter of neurosecretory cells increases slightly (19.8 µm) while the number increases up to 59.5 cells per ganglion (Fig. 3). In four-month-old snails, the neurosecretory cells occur in two or three layers and the mean diameter and number of neurosecretory cells increase to 21 µm (Fig. 3A) and 99 cells per ganglion (Fig. 3B), respectively.

In five-month-old snails, the neurosecretory cells contain numerous small vacuoles (Fig. 1C) and they differ in size within a cluster, with the largest cells located in the most peripheral layer. The mean diameter of neurosecretory cells in five-month-old snails is 21 µm (Fig. 3A). The number of neurosecretory cells increases to 107 cells per ganglion (Fig. 3B).

The mean diameter of neurosecretory cells continues to increase slightly in six to twelve-month-old snails (Fig. 3A). The number of neurosecretory cells continues to increase in six- to eight-month-old snails. It reaches to a peak in eight-month-old snails. The number then decreases in nine-month-old snails but remains constant in nine- to twelve-month-old snails (Fig. 3B).

In some neurosecretory cells of six to eight-month-old snails, the small vacuoles have fused to form a single large vacuole which appears to accumulate some substance which stains pink or red with phloxine. Other cells, however, still contain several small vacuoles. In nine to twelve-month-old snails, all the neurosecretory cells in the cerebral ganglia contain a round nucleus and a single large vacuole (Fig. 1D).

The transmission electron microscope reveals that the neurosecretory cells are closely attached to the perineurium or surrounding connective tissue of the cerebral ganglion. The nucleus of neurosecretory cells is round and heterochromatic (Fig. 2A). There is a conspicuous single large vacuole in the cell (Fig. 2A). In the cytoplasm, the cisternae of rough endoplasmic reticulum are highly elaborate and arranged in many arrays (Fig. 2B). The electron-dense elementary granules with a mean diameter of 1600 A° are the prominent inclusions found in the cytoplasm (Fig. 2B). These granules are intermingled with microtubules and numerous mitochondria (Fig. 2C). Moreover, there are many groups of Golgi apparatus, each consists of many saccules (Fig. 2D). Lysosomes are also numerous in the cytoplasm of these neurosecretory cells (Fig. 2D).

Discussion

The occurrence of neurosecretory cells has been investigated in many species of pulmonate snails, *i.e.*, *L. stagnalis* (Joosse, 1964; Boer, 1965), *A. glabratus* (Lever *et al.*, 1965), *H. tenue* (Simpson *et al.*, 1966), *H. duryi* (Wetherby) (Dillaman *et al.*, 1976) and *A. ater* (Smith, 1967), usually with chrome-hematoxylin phloxine and paraldehyde-fuchsin staining methods (Gomori, 1941; 1950). In the present study, these two methods were chosen to study the development of neurosecretory cells in the cerebral ganglia of *A. fulica*.

Postembryonic development of neurosecretory cells in the cerebral ganglia showed that they first appear in two-month-old snails. A large number of neurosecretory cells is found in eight-month-old snails which apparently is correlated with the growth of the snails. These neurosecretory cells occurred in constant and specific areas.
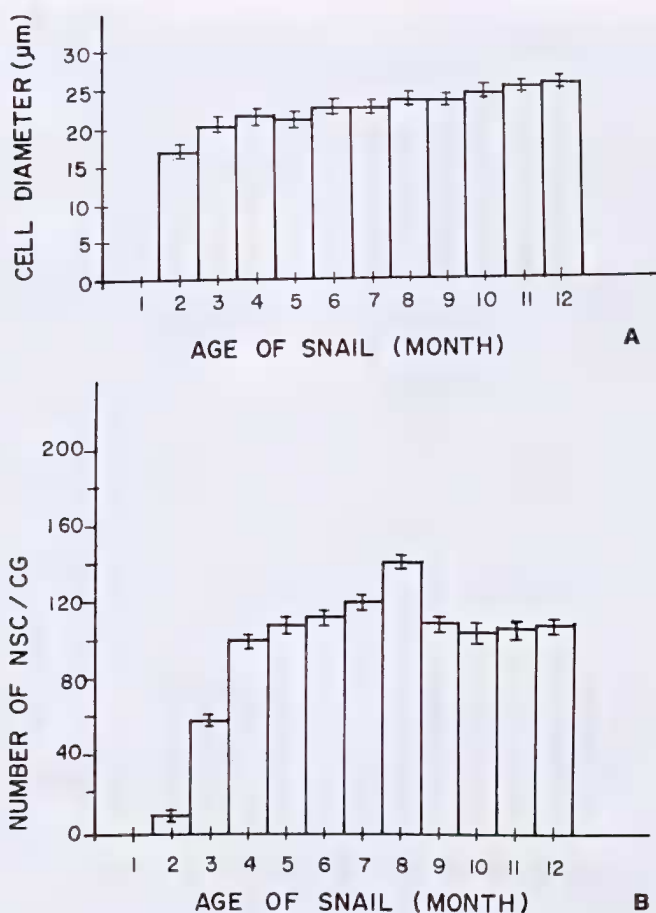
Figure 3. A. The mean diameter of neurosecretory cells in cerebral ganglia of *Achatina fulica* at various ages. B. The number of neurosecretory cells (NSC) in cerebral ganglia (CG) of *Achatina fulica* at various ages.

There are many reports on the functions of neurosecretory cells in the cerebral ganglia of basommatophoran and stylommatophoran snails. The light green cells in the cerebral ganglia of *L. stagnalis*, and the medial cells in the cerebral ganglia of *Agriolimax reticulatus* (Müller) have a factor that stimulates growth rate (Geraerts & Algera, 1976; Widjenes & Runham, 1977). The caudo-dorsal cells, found only in *L. stagnalis*, affect ovulation and egg-laying behavior (Geraerts & Bohlken, 1976). However, in stylommatophoran snails, there is a substance that stimulates the movement of oocytes. Saleuddin *et al.* (1983) reported that the brain extract (cerebral ganglia with their capsule) caused the amoeboid movement of oocytes in *H. aspersa*.

The development of neurosecretory cells in the cerebral ganglia of *A. fulica* involves two processes, increases in cell size and cell number. The development of these neurosecretory cells may be correlated to the development of the reproductive organs. Ngowsiri *et al.* (1988) reported that the oocytes first appear in the ovotestis of *A. fulica*

at the age of about five months. At this stage, there are many large neurosecretory cells in the cerebral ganglia. The snails reach sexual maturity in five to seven months (Bequaert, 1950; Pawson & Chase, 1984; Upatham *et al.*, 1988; Mohr & Meer, 1949; Kondo, 1964).

Ngowsiri *et al.* (1988) reported that in *A. fulica*, the number of oocytes progressively increased as the snails approached the age of 7–8 months, and in the present study, the number of neurosecretory cells is shown to increase at a similar rate. It thus may be suggested that the neurosecretory cells in the cerebral ganglia of *A. fulica* have a direct effect on the production of oocytes in the ovotestis.

The ultrastructure of the neurosecretory cells is indicative of high synthetic activity. There are electron-dense elementary granules which occur in association with well developed rough endoplasmic reticulum and Golgi apparatus. In the active medio-dorsal cells and latero-dorsal cells of the cerebral ganglia of *L. stagnalis*, the Golgi complex is extremely large (Boer, 1965). The Golgi lamellae contain electron-dense elementary material which is formed into electron-dense elementary granules by budding (Boer, 1965). A similar event also occurs in the neurosecretory cells in the cerebral ganglia of *A. fulica*.

The cytoplasm of neurosecretory cells in the cerebral ganglia of *A. fulica* contains abundant electron-dense elementary granules, with a mean diameter of 1,600 A°. Electron-dense elementary granules (1,000–3,000 A°) containing neurohormones have been described in "Gomori-positive" cells in various species of snails (Boer *et al.*, 1965; Lever *et al.*, 1965; Simpson *et al.*, 1966; Wendelaar Bonga, 1970).

The presence of a large vacuole in the cell is another characteristic of the neurosecretory cells in the cerebral ganglia of *A. fulica*. These vacuoles, as observed with the light microscope, usually contain pink or red substance when stained with chrome-hematoxylin phloxine. No vacuole is present in ordinary neurons. These vacuoles might be intracellular storage of neurohormones similar to the lumen of thyroid cells in vertebrates (Nadler *et al.*, 1962).

The transport of neurosecretory material *in vivo* has not been critically studied but a few workers suggested that microtubules might be involved (Loh *et al.*, 1975; Roubos, 1975). In *A. fulica*, there are numerous microtubules dispersed in the area of electron-dense elementary granules, which may function in the transport of neurosecretory material. In *H. aspersa*, the localization of electron-dense elementary granules along the periphery of neurosecretory cells adjacent to the connective tissue and the presence of dense clusters of granules in the connective tissue indicates the release of neurosecretory material (Kai-Kai & Kerkut, 1979). Wendelaar Bonga (1970) reported the peripheral release of neurosecretory products in *L. stagnalis*. In *A. fulica*, the neurosecretory cells are closely attached to the perineurium or connective tissue of the cerebral ganglia. Hence, it may be suggested that the neural sheath of the cerebral ganglia might be the neurohaemal area of neurosecretory cells.

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